

Claims

5

15

- 1. A purified thermostable DNA polymerase obtainable from *Thermococcus gorgonarius* which catalyses the template directed polymerisation of DNA, possesses 3'-5'-exonuclease (proofreading) activity and is characterised by at least a two-fold greater replication fidelity than DNA polymerase obtainable from *Pyroccoccus furiosus*.
- 2. A purified thermostable DNA polymerase according to claim 1 which retains about 90 % of its activity after incubation for two hours at about 95°C in the presence of a stabilizer.
- 3. The polymerase as claimed in any one of claims 1 2, wherein said polymerase has an apparent molecular weight between about 92 000 to 96 000 daltons.
 - 4. The polymerase as claimed in any one of claims 1 3, wherein said polymerase is obtainable from <u>E. coli</u>.
 - 5. A stabilized composition consisting of a polymerase as claimed in any one of claims 1 4 and a stabilizer.
 - 6. The composition according to claims 1 5, wherein said stabilizer is a non-ionic detergent.
 - 7. The composition according to claim 1 6, wherein Thesit and/or Nonident P 40 serve as stabilizer.
- 8. An isolated DNA sequence coding for the polymerase according to any one of claims 1 7

 obtainable from *Thermococcus gorgonarius*.
 - 9. An isolated DNA sequence coding for the polymerase as claimed in claim 8 contained within the plasmid pBTac2Tgo.
 - 10. An isolated DNA sequence of claim 9 contained within an approximately 2.3 kB EcoRI/Pstl restriction fragment of plasmid pBTac2Tgo.
- 25 11. An isolated DNA sequence represented by the formula shown in SEQ ID No. 6.
 - 12. A vector containing the isolated DNA sequence as claimed in any one of claims 8 11.
 - 13. The vector of claim 12, wherein such vector is plasmid pBTac2Tgo.
 - 14. The vector according to claims 12 and 13 providing some or all of the following features:

 (1) promotors or sites of initiation of transcription

10

15

20

- (2) operators which could be used to turn gene expression on or off
- (3) ribosome binding sites for improved translation
- (4) transcription or translation termination sites.
- 15. A microbial host transformed with the vector of claims 12 14.
- 5 16. A microbial host according to claim 15 wherein said host is from *E. coli* LE 392 pUBS 520 and designated *E.coli* pBtac2Tgo.
 - 17. A process for the preparation of DNA polymerase according to any one of claims 1 7 comprising the steps:
 - (a) cultering the natural strain Thermococcus gorgonarius
 - (b) suspending the cells of the natural cells in buffer
 - (c) disrupting the cells
 - (d) purifying the DNA polymerase by several chromatographic steps.
 - 18. A process for the preparation of DNA polymerase according to any one of claims 1 7 comprising growing a microbial host strain according to claims 15 or 16 and purifying the DNA polymerase therefrom.
 - 19. A process for amplifying DNA, characterized in that a thermostable DNA polymerase according to any one of claims 1 7 is used.
 - 20. A process for DNA sequencing or DNA labelling, characterized in that a thermostable DNA polymerase according to any of the claims 1 7 is used wherein the 3'-5' exonuclease activity of said DNA polymerase is inactivated.
 - 21. A process for second cDNA cloning and DNA sequencing, characterized in that a thermostable DNA polymerase according to any one of claims 1 7 is used.
 - 22. A process for DNA sequencing, characterized in that a thermostable DNA polymerase according to any one of claims 1 7 is used.